



**[0006]** The invention is based on the object of creating a method with which shading effects of the lens of a microscope system can be eliminated.

**[0007]** The envisaged object is achieved by a method having the features of claim 17.

**[0008]** It is advantageous for the microscope system to be provided with at least one lens that is present in the microscope system and that defines an illumination field. Furthermore, at least one light source is provided in the microscope system so as to emit an illuminating light beam that illuminates a specimen through the lens. By the same token, at least one detector is present that, pixel-by-pixel, detects a detection light beam coming from the specimen. An electronic circuit downstream from the detector serves to process the image data captured by the detector. A wavelength-dependent brightness distribution of the illumination field of the lenses present in the microscope system is saved in a memory unit. An actuatable element is provided in the illuminating light beam and it controls the intensity of the illuminating light beam pixel-by-pixel as a function of the stored, wavelength-dependent brightness distribution in such a way that the illumination field is homogeneously illuminated. The electronic circuit computes the saved wavelength-dependent brightness distribution pixel-by-pixel in such a way that a homogeneously illuminated image field is formed.

**[0009]** The actuatable element in the illuminating light beam is an LCD matrix whose individual pixels are actuated according to the stored, wavelength-dependent brightness distribution. In one embodiment, the detector is a CCD chip.

**[0010]** In another embodiment, a scanning device is provided in the illuminating light beam of the microscope system and it conducts the illuminating light beam pixel-by-pixel over or through the specimen. The actuatable element in the illuminating light beam is an acousto-optic element that can be actuated as a function of the wavelength-dependent brightness distribution saved in the memory unit in such a way that the illumination field consisting of the individual pixels has a homogeneous brightness

distribution. The acousto-optic element is an AOTF (Acousto-Optic Tunable Filter) or an AOBS (Acousto-Optic Beam Splitter) or an AOM (Acousto-Optic Modulator).

**[0011]** The microscope system can be equipped with different light sources such as, for example, a laser, a multiline laser or a laser that emits a continuous wavelength spectrum.

**[0012]** If a laser is used with the scanning device, the detector of the microscope system comprises at least one light-sensitive element that serially captures the pixels of the illumination field on the specimen. The electronic circuit combines the individual pixels to form an image field that can be computed with the appropriate wavelength-dependent brightness distribution.

**[0013]** The method for the shading correction is characterized by the following steps:

- saving the wavelength-dependent brightness distribution in a RAM table;
- pixel-by-pixel actuation of an element with a wavelength-dependent brightness distribution of the illumination field of the lens in such a way that the illumination field is homogeneously illuminated;
- pixel-by-pixel detection of the detection light beam coming from the specimen; and
- employing the wavelength-dependent brightness distribution of the illumination field of the lens in order to compute the image field captured with the lens.


**[0014]** Additional advantageous embodiments of the invention can be gleaned from the subordinate claims.

**[0015]** The subject matter of the invention is schematically shown in the drawing and it is described below with reference to the figures, which show the following:

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- Figure 1 a schematic depiction of a microscope with an actuatable element in the illuminating light beam;
- Figure 2 a schematic depiction of a scanning microscope with an actuatable element in the illuminating light beam;
- Figure 3 a schematic depiction of another embodiment of the scanning microscope with a control circuit for changing the intensity of the illuminating light beam coming from the light source;
- Figure 4a a schematic depiction of the brightness distribution for an image field;
- Figure 4b a schematic depiction of the brightness distribution for an illumination field;
- Figure 5 a schematic depiction of a surface sensor for capturing the detection light beam coming from the specimen; and
- Figure 6 a schematic depiction of the actuatable element with which the individual pixels are actuated according to the brightness distribution for a wavelength.

**[0016]** Figure 1 schematically describes a microscope system 1. The embodiment of the microscope system shown here comprises a reflected light microscope. It goes without saying that the microscope system 1 can likewise comprise a transmitted light microscope or a configuration with which it is possible to switch between both types of light. The schematic depiction of the microscope only shows those components that are essential to the description. All of the other elements or components such as, for example, the stand, the revolving nosepiece, the tube, the eyepiece, the camera, the camera tube, etc. are sufficiently familiar to a person skilled in the art and hence do not have to be mentioned here explicitly. The microscope comprises at least one light source 3 that emits an illuminating light beam 5 that is depicted in Figure 1 as a solid line. In the

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embodiment shown here, a beam deflection means 7 is provided that deflects the illuminating light beam 5 onto a lens 9 located in the working position. The lens 9 is positioned above a specimen 10 that, in turn, is located on a microscope slide 11. It is sufficiently familiar to a person skilled in the art that the microscope slide 11 can be positioned on a movable stage. For the sake of simplicity, the stage is not shown here. The embodiment shown here is a reflected light microscope so that the light coming from the specimen 10 is imaged by the lens 9 onto at least one detector 20. The detector 20 and the lens 9 are arranged in the detection light beam 12. The detection light beam 12 is shown as a broken line. In the case of a lens, a so-called lens system, if one leaves aside all aberrations such as, for instance, astigmatism, longitudinal chromatic aberrations, transverse chromatic aberrations, etc., then only one curved surface is always imaged onto another curved surface. This is also referred to as image field curvature. This phenomenon applies to individual objectives as well as to entire microscope systems and leads to a shading effect in the captured images. Figure 4a shows an image field 40. Each image field 40 becomes darker and darker as measured starting from a mid-point 41, that is to say, the intensity of the captured image field 40 or else the intensity of the illumination field decreases from the mid-point 41 towards the outside. This drop in intensity from the mid-point 41 towards the outside is likewise dependent on the wavelength. The image field 40 is, for example, rectangular and the intensity decreases towards a first and second long sides 42a and 42b respectively, and towards a first and second short sides 43a and 43b. An actuatable element 13 is provided in the illuminating light beam 5 and in the detection light beam 12. The actuatable element 13 is connected to an electronic circuit 14 that is provided with a memory unit 15. A wavelength-dependent brightness distribution such as, for example, in Figure 4a of the illumination field of the lenses 9 present in the microscope system is saved in the memory unit 15. By the same token, the electronic circuit 14 can also be connected to the lens 9 or to the revolving nosepiece, so that the electronic circuit 14 continuously receives information about the lens 9 that is momentarily located in the illuminating light beam or in the detection light beam. It is clear to any person skilled in the art that different lenses exhibit different shading. The electronic circuit 14 serves to control the intensity of the illuminating light beam 4 pixel-by-pixel as a function of the stored wavelength-dependent


brightness distribution. The actuatable element 13 is controlled with the stored wavelength-dependent brightness distribution in such a way that the illumination field 46 (see Figure 4b) is homogeneous and does not display any drop in the intensity towards the long sides 42a, 42b and/or towards the short sides 43a and 43b. The detection light beam 12 coming from the specimen 10 is computed with the wavelength-dependent brightness distribution stored in the memory unit 15 in such a way that a homogeneously illuminated image field 40 is created. The detection light beam 12 coming from the specimen is detected pixel-by-pixel by the detector 20, which is a CCD chip. This data is fed to the electronic circuit 14 which then compares the stored wavelength-dependent brightness distribution to the measured wavelength-dependent brightness distribution as a function of the wavelength.

**[0017]** Figure 2 shows the schematic structure of a scanning microscope 100 in which the idea according to the invention is implemented. The illuminating light beam 5 coming from at least one light source 3 is directed at an actuatable element 13. The illuminating light beam 5 coming from the actuatable element 13 is conducted to a scanning device 16. In the embodiment disclosed here, the scanning device 16 comprises a gimbal-mounted scanning mirror 18 that conducts the illuminating light beam 3 through a scanning lens 19 and through a lens 9 over or through a specimen 10. In case of a non-transparent specimen 10, the illuminating light beam 5 is conducted over the specimen surface. In the case of biological specimens 10 (preparations) or transparent specimens 10, the illuminating light beam 5 can also be conducted through the specimen 10. In order to increase the contrast, non-luminous preparations can optionally be prepared with a suitable dye (not shown, since this is the established state of the art). The dyes present in the specimen 10 are excited by the illuminating light beam 5 and emit light in a characteristic spectral range that is specific to them. This light coming from the specimen defines a detection light beam 12. It passes through the lens 9, the scanning lens 19 and, via the scanning device 16, reaches the actuatable element 13, traverses the latter without being influenced and, for example, via a detection pinhole 21, reaches at least one detector 20 that is configured as a photomultiplier. It is clear to the person skilled in the art that other detection components such as, for instance, diodes, diode arrays,

photomultiplier arrays, CCD chips or CMOS image sensors can also be used. The detection light beam 12 coming from or defined by the specimen 10 is shown in Figure 2, like in Figure 1, as a broken line. Electric detection signals that are proportional to the output of the light coming from the specimen 10 are generated in the detector 20. Since, as already mentioned above, the specimen 10 does not emit light of only one wavelength, it is advantageous to install a selection means for the spectrum coming from the sample 10 upstream from the at least one detector 20. In the embodiment shown here, the selection means is an SP module 23. The SP module 23 is configured in such a way that it can capture an entire lambda scan, that is to say, that all of the wavelengths emitted by the light source 3 can be recorded. By the same token, several of the wavelengths coming from the specimen 10 can be spatially separated and, if applicable, can also be recorded parallel in time. The data generated by the detector 20 is forwarded to the electronic circuit 14. At least one peripheral device 27 is associated with the electronic circuit 14. This peripheral device 27 can be, for example, a display on which the user receives information about the setting of the scanning microscope or about the current setup and can also obtain the image data in graphic form. Moreover, the memory unit 15 is connected to the electronic circuit 14 and, as already described, this is where the wavelength-dependent brightness distribution – shown, for example, in Figure 3b – of the illumination field 46 of the lenses 9 present in the microscope system 1 is saved.

**[0018]** The SP module 23 spatially, spectrally splits the detection light beam 12 with a prism 31. Another possibility for spectral splitting is the use of a reflecting diffraction grating or a transmission diffraction grating. The spectrally split light fan 32 is focused with the focusing lens 33 and subsequently strikes a mirror diaphragm arrangement 34, 35. The mirror diaphragm arrangement 34, 35, the means for spectral, spatial splitting, the focusing lens 33 and the detectors 36 and 37 are referred to together as the SP module 23 (or multi-band detector).


**[0019]** The scanning device 16 conducts the illuminating light beam 5 pixel-by-pixel over or through the specimen 10. The actuatable element 13 in the illuminating light beam 5 is an acousto-optic element that can be actuated as a function of the wavelength-

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dependent brightness distribution saved in the memory unit 15 in such a way that the illumination field 46 consisting of the individual pixels has a homogenous brightness distribution. The acousto-optic element 13 is an AOTF (Acousto-Optic Tunable Filter) or an AOBs (Acousto-Optic Beam Splitter) or an AOM (Acousto-Optic Modulator). The light source 3 consists of at least one laser that generates the illuminating light beam 5. The at least one laser can be a multiline laser. By the same token, it is conceivable for the laser to emit a continuous wavelength spectrum so that the specimen 10 is either illuminated with a continuous wavelength spectrum or the user selects any desired wavelengths from the continuous wavelength spectrum in order to illuminate the specimen 10.

**[0020]** Figure 3 shows a schematic depiction of another embodiment of the scanning microscope 100 with a control circuit 60 for changing the intensity of the illuminating light beam 5 coming from the light source 3. The same elements as already described for Figure 2 are designated with the same reference numerals. The light source 3, which is configured as a multiline laser, is provided with the control circuit 60 so that the intensity of the illumination light being emitted by the laser is controlled as a function of the stored wavelength-dependent brightness distribution. The illumination field 46 (see Figure 4b) is then illuminated homogeneously and does not exhibit any drop in the intensity towards the long sides 42a, 42b and/or towards the short sides 43a, 43b. The dyes present in the specimen 10 are excited by the illuminating light beam 5 and emit light in a characteristic spectral range that is specific to them. This light coming from the specimen defines a detection light beam 12. It passes through the lens 9, the scanning lens 19 and, via the scanning device 16, reaches a wavelength-selective element 63, traverses the latter without being influenced and, for example, via a detection pinhole 21, reaches at least one detector 20 that is configured as a photomultiplier. Other embodiments of the detector were already mentioned in the description pertaining to Figure 2.

**[0021]** Figure 5 shows a schematic depiction of the detector 20 which, in this embodiment, is configured as an area sensor 44 in order to detect the detection light beam 12 coming from the specimen 10. The area sensor 44 comprises multiple pixels 45<sub>1,1</sub>,

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$45_{1,2}$ ,  $45_{1,3}$ , ...,  $45_{n,m-1}$ ,  $45_{n,m}$ , which are arranged on a surface. The light coming from the specimen 10 is imaged by the lens 9 onto the area sensor 44. The individual pixels  $45_{1,1}$ ,  $45_{1,2}$ ,  $45_{1,3}$ , ...,  $45_{n,m-1}$ ,  $45_{n,m}$  register the intensity, which is superimposed on the shading effect of the lens 19. The wavelength-dependent brightness distribution is saved in the memory unit 15 and the electronic circuit 14 compares the data captured by the detector 20 to the wavelength-dependent brightness distribution, so that the captured image field 40 is homogeneously illuminated at the wavelength  $\lambda_n$ .

**[0022]** Figure 6 shows a schematic depiction of the actuatable element 13 where the individual pixels  $50_{1,1}$ ,  $50_{1,2}$ ,  $50_{1,3}$ , ...,  $50_{n,m-1}$ ,  $50_{n,m}$  are actuated according to the brightness distribution for a given wavelength. Generally speaking, the gray values associated with the pixels  $50_{1,1}$ ,  $50_{1,2}$ ,  $50_{1,3}$ , ...,  $50_{n,m-1}$ ,  $50_{n,m}$  constitute the shading effect caused by the lens 9. This is the negative depiction for the actuation of the individual pixels  $50_{1,1}$ ,  $50_{1,2}$ ,  $50_{1,3}$ , ...,  $50_{n,m-1}$ ,  $50_{n,m}$ . This means that the pixels  $50_{1,1}$ ,  $50_{1,2}$ ,  $50_{1,3}$ , ...,  $50_{n,m-1}$ ,  $50_{n,m}$  are actuated inversely as a function of the gray value, that is to say, the darker the pixels  $50_{1,1}$ ,  $50_{1,2}$ ,  $50_{1,3}$ , ...,  $50_{n,m-1}$ ,  $50_{n,m}$  are, the less the shading caused by the actuation is. As already mentioned, the actuation of the actuatable element 13 is such that the illumination field 46 on the specimen 10 is homogeneously illuminated.

**[0023]** A laser beam illuminates the specimen 10 in a meander-like manner, so that the specimen 10 is illuminated, that is to say, the illumination field is covered consecutively with a plurality of image points. A scanning device 16 conducts the illuminating light beam 5 pixel-by-pixel over or through the specimen 10 in order to yield a homogeneously illuminated illumination field 46; the actuatable element 13 in the illuminating light beam 5 is an acousto-optic element 13. As shown in the depiction of Figure 5, the acousto-optic element 13 is likewise actuated inversely. This means that the greater the gray value due to the shading is, the higher the intensity of the laser beam has to be when it reaches this position. This takes place as a function of the wavelength-dependent brightness distribution saved in the memory unit 15 in such a way that the illumination field 46 consisting of the individual pixels has a homogenous brightness distribution.

